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Inhibition of Ape1 Nuclease Activity by Lead, Iron, and Cadmium

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Many environmental metals are co-carcinogens, eliciting their effects via inhibition of DNA repair. Apurinic/apyrimidinic (AP) endonuclease 1 (Ape1) is the major mammalian abasic endonuclease and initiates repair of this cytotoxic/mutagenic lesion by incising the DNA backbone via a Mg^{2+}-dependent reaction. In this study we examined the effects of arsenite [As(III)], cadmium [Cd(II)], cobalt [Co(II)], iron [Fe(II)], nickel [Ni(II)], and lead [Pb(II)] at concentrations ranging from 0.3 to 100 μM on the incision activity of Ape1 in the presence of 1 mM $MgCl_2$. Pb(II) and Fe(II) inhibited Ape1 activity at each of the concentrations tested, with an IC_{50} (half-maximal inhibitory concentration) of 0.61 and 1.0 μM, respectively. Cd(II) also inhibited Ape1 activity but only at concentrations > 10 μM. No inhibition was seen with As(III), Co(II), or Ni(II). A similar inhibition pattern was observed with the homologous *Escherichia coli* protein, exonuclease III, but no inhibition was seen with the structurally distinct AP endonuclease *E. coli* endonuclease IV, indicating a targeted effect of Pb(II), Fe(II), and Cd(II) on the Ape1-like repair enzymes. Excess nonspecific DNA did not abrogate the metal inactivation, suggesting a protein-specific effect. Notably, Cd(II), Fe(II), and Pb(II) [but not As(III), Co(II), or Ni(II)] inhibited AP endonuclease activity in whole-cell extracts but had no significant effect on single nucleotide gap filling, 5′-flap endonuclease, and nick ligation activities, supporting the idea of selective inactivation of Ape1 in cells. Our results are the first to identify a potential DNA repair enzyme target for lead and suggest a means by which these prevalent environmental metals may elicit their deleterious effects. *Key words:* Ape1 AP endonuclease, base excision DNA repair, environmental heavy metal toxicity, lead, mutagenesis/carcinogenesis. *Environ Health Perspect* 112:799–804 (2004). doi:10.1289/txg.7038 available via *http://dx.doi.org/* [Online 13 April 2004]

Toxic metal compounds are widely distributed in the environment and are frequently used in industrial processes (Hayes 1997). Because of their extended persistence in biological systems and their tendency to accumulate in certain tissues, they represent important environmental and occupational hazards. Epidemiologic studies and animal trials have shown many metal compounds to be carcinogenic, although they elicit only mild mutagenic effects in bacterial test systems or in mammalian cell culture (Hartwig et al. 2002; Hartwig and Schwerdtle 2002).

DNA repair systems function to correct DNA damage that arises spontaneously or due to exposure to certain environmental agents (Hoeijmakers 2001). It is well documented that reduced DNA repair capacity can lead to genetic instability and thus human disease, most notably cancer. Because cell biology studies indicate that many metal compounds enhance the genotoxic effects of known mutagens such as ultraviolet C radiation, X rays, benzo[*a*]pyrene, cisplatin, and DNA alkylating agents, it has been postulated that environmental metals are comutagenic and potentiate the carcinogenic effects of DNA-damaging agents by inhibiting DNA repair processes (Hartwig et al. 2002; Hartwig and Schwerdtle 2002; Hayes 1997). Consistent with this notion, both *in vitro* and *in vivo* studies have shown that certain metal compounds can inhibit specific DNA repair events.

For example, Cd(II), Cu(II), Co(II), and Ni(II) inhibit (at concentrations ≥ 200 μM) the *in vitro* DNA-binding activity of the human xeroderma pigmentosum group A protein, a critical damage recognition factor in nucleotide excision repair (Asmuss et al. 2000). Poly(adenosine diphosphate-ribose)polymerase 1, which operates as a molecular sensor in DNA strand-break responses, is inactivated by Ni(II), Co(II), Cd(II), Cu(II), and very low concentrations (10 nM) of As(III) (Hartwig et al. 2003). The DNA-binding capacity of the tumor suppressor protein p53 is impaired by Cd(II), Ni(II), and Co(II) (Meplan et al. 1999; Palecek et al. 1999). In addition, Cd(II) and Zn(II) (at ≥ 100 μM) inactivate the *in vitro* DNA glycosylase activity of Ogg1, a mammalian repair enzyme that functions to excise damaged/mutagenic bases, such as 8-oxoguanine, from DNA (Zharkov and Rosenquist 2002).

Cd(II) was recently shown to inhibit DNA mismatch repair (MMR) (Jin et al. 2003), a process known to correct replication errors; deficiencies in this pathway have been linked to the development of hereditary nonpolyposis colorectal cancer (Heinen et al. 2002). Although the precise target for MMR inhibition remains unclear, Jin et al. (2003) found that yeast chronically exposed to environmental concentrations of Cd(II) display an increased mutation rate genetically dependent on an intact MMR pathway. Thus, studies are beginning to unveil the molecular targets of environmental metals and the mechanisms by which they may elicit their comutagenic effects and, hence, carcinogenic potential.

Apurinic/apyrimidinic (AP) sites are frequent lesions in DNA, generated by spontaneous, damage-induced, or enzyme-catalyzed hydrolysis of the *N*-glycosylic bond, which attaches the base moiety to the sugar residue (Wilson and Barsky 2001). If unrepaired, these noncoding lesions present both cytoxic and mutagenic challenges to the cell. AP endonuclease 1 (Ape1) is the major mammalian abasic endonuclease, accounting for > 95% of the total cellular AP site incision activity (Demple and Harrison 1994). This enzyme initiates repair of AP sites by cleaving the phosphodiester backbone 5′ to the damage site, a critical step in the base excision repair (BER) pathway, which handles most spontaneous, alkylation, and oxidative DNA damage (Kelley et al. 2003). In addition to its role as an AP endonuclease, Ape1 functions in specific strand-break contexts to excise 3′-oxidative blocking termini (e.g., phosphoglycolate and phosphate damages) (Suh et al. 1997; Wilson 2003; Winters et al. 1994), as well as certain 3′-mismatched nucleotides (Chou and Cheng 2002; Hadi et al. 2002), via its 3′- to 5′-phosphodiesterase/exonuclease activity. Each of these Ape1 repair functions is carried out by the same metal (magnesium)-dependent catalytic reaction (Gorman et al. 1997; Mol et al. 2000b).

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Animal studies have shown that heterozygous (reduced-function) *APE1* mice, which maintain 50% Ape1 repair activity, exhibit increased risk for phenotypic consequences associated with oxidative stress, most notably reduced survival and elevated cancer susceptibility (Meira et al. 2001). Because both X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy studies revealed that lead can bind the active site of Ape1 (Beernink et al. 2001; Lowry et al. 2003), we explored the effects of several heavy metals, at presumed biological concentrations, on Ape1 DNA repair function. In this article, we demonstrate that Pb(II) and Fe(II), and to a lesser degree Cd(II), inhibit Ape1 nuclease activity, unveiling a novel molecular target for these environmental metals.

Materials and Methods

Reagents

Recombinant, untagged human Ape1, *Escherichia coli* exonuclease III (ExoIII), and *E. coli* endonuclease IV (EndoIV) proteins were purified essentially as described (Erzberger et al. 1998). DNA oligonucleotides were obtained from The Midland Certified Reagent Company (Midland, TX) [see Figure 1 and Wilson (2003) for details]. All heavy metal compounds were purchased from Sigma-Aldrich (St. Louis, MO). Denaturing polyacrylamide gel materials were obtained from National Diagnostics (Atlanta, GA).

Ape1 Nuclease Assays

Ape1 incision activity was monitored essentially as described (Wilson et al. 1995). Briefly, the tetrahydrofuran (F)-containing oligonucleotide was 5′-^{32}P-end labeling and annealed to the complementary DNA (see Figure 1A for nucleotide sequences). AP endonuclease reactions (unless otherwise instructed) consisted of the following: 50 mM Hepes, pH 7.5; 100 mM KCl; 1 mM $MgCl_2$; 200 pg (0.56 nM, assuming a molecular weight of 35,555 g/mol) Ape1, and either 0, 0.3, 1, 3, 10, 30, or 100 μM As(III), Cd(II), Co(II), Fe(II), Ni(II), or Pb(II) (as indicated) in a final volume of 10 μL. After a 10-min incubation on ice, 1 pmol labeled DNA substrate was added. Incision reactions were performed at 37°C for 10 min, stopped by the addition of 10 μL formamide buffer, and analyzed by electrophoresis on an 18% denaturing polyacrylamide gel. AP endonuclease activity is the amount of starting radiolabeled tetrahydrofuran containing DNA (F-DNA) substrate converted to the shorter incised DNA product in 10 min. Percent conversion was determined using standard Molecular Dynamics phosphorimager analysis and ImageQuant, version 5.2, software (Amersham Biosciences Corp., Piscataway, NJ).

For the nonspecific competitor DNA assays, everything was identical except labeled F-DNA and Ape1 were present at 0.1 pmol and 50 pg, respectively. After incubation of Ape1 with the metal ion, F-DNA was added with either 0.1 or 1 pmol of undamaged, duplex (26-mer) competitor DNA. Incision reactions were performed immediately, and the samples were analyzed as indicated above.

Endonuclease IV/Exonuclease III Assays

EndoIV reactions consisted of 100 mM Hepes, pH 7.5; 100 mM KCl; 20% glycerol; 0.02% TX-100; 1 ng (3.2 nM, assuming a molecular weight of 31,480 g/mol) EndoIV, and 100 μM of the indicated metals in 10 μL. ExoIII reactions consisted of 50 mM Hepes, pH 7.5; 100 mM KCl; 1 mM $MgCl_2$; 1 ng (3.2 nM, assuming a molecular weight of 30,969 g/mol) ExoIII, and 100 μM of the appropriate metals (as indicated) in 10 μL. DNA substrate (1 pmol) was added after a 10-min incubation on ice, and the incision efficiency was determined as for Ape1 (see above).

Whole-Cell Extract Assays

293T cells (80% confluent) were trypsinized and washed with phosphate-buffered saline. The cells were then frozen at –80°C for 1 hr before cell extract production. Cells were resuspended in 1 mL lysis buffer (50 mM Tris, pH 7.4, 1 mM EDTA, 1 mM DTT, 10% glycerol, 0.5 mM PMS) and sonicated, and a Bradford assay was run to determine the protein concentration of the supernatant (whole-cell extract).

AP endonuclease, single nucleotide (nt) gap-filling, 5′-flap endonuclease, and nick ligation activities were determined using the oligonucleotide substrates described in Figure 1B. For the first three activities, reaction conditions were the same as that used in the Ape1 assays above. The gap-filling reaction also included 100 μM dCTP to monitor nucleotide incorporation. One microgram of whole-cell extract was used to measure AP endonuclease activity, 10 μg for flap endonuclease activity, and 20 μg for gap-filling activity, in a final volume of 10 μL. Nick ligation reactions consisted of 60 mM Tris-HCl, pH 8.0; 10 mM $MgCl_2$; 1 mM DTT; 50 μg/mL BSA; 1 mM ATP; and 30 μg cell extract in a final volume of 20 μL. Whole-cell extracts were incubated (where indicated) for 10 min on ice with 100 μM As(III), Cd(II), Co(II), Fe(II), Ni(II), or Pb(II). After incubation, 1 pmol of the appropriate DNA substrate was added, and reactions were performed as follows: AP endonuclease, gap-filling, and flap endonuclease activities for 10 min at 37°C; and nick ligation assays for 30 min at room temperature. The percentage of substrate

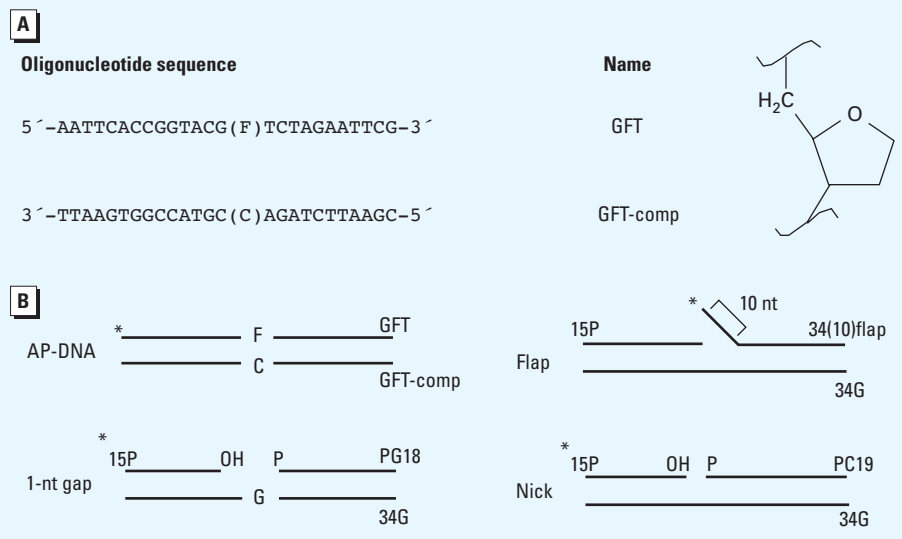

Figure 1. Oligonucleotide substrates. C, cytosine; comp, complementary DNA; F, tetrahydrofuran; G, guanine; GFT, guanine–tetrahydrofuran–thymine-containing 26-mer oligonucleotide; nt, nucleotide; OH, hydroxyl; P, phosphate. Numbers indicate oligonucleotide length. (*A*) Nucleotide sequence of the 26-mer AP–DNA duplex and the chemical composition of the synthetic abasic residue. F represents the abasic site analog tetrahydrofuran. (*B*) Schematic of the oligonucleotide substrates used to measure AP endonuclease (AP–DNA), 1-nt gap-filling (1-nt gap), 5′-flap endonuclease (flap), and nick ligation (nick) activities. Further details of these substrates can be found in *A* or Wilson 2003). The position of the radiolabel is indicated by the asterisk. The nucleotide sequence of 34(10)flap is as follows: 5′-GGTAGGTAAACGTACG-GATCCCCGGGTAC-3′.

converted to product was determined after denaturing polyacrylamide gel electrophoresis using standard phosphorimager analysis as above.

Results

Inhibition of Ape1 Nuclease Activities by Lead, Iron, and Cadmium

Using a 26-mer oligonucleotide duplex containing a single, centrally located abasic site analog—the F residue (Figure 1A)—we assessed the effects of several common environmental metals on Ape1 incision capacity. In brief, Ape1 was incubated in reaction buffer containing 1 mM $MgCl_2$ with 0, 0.3, 1, 3, 10, 30, or 100 μM As(III), Cd(II), Co(II), Fe(II), Ni(II), or Pb(II). After incubation on ice for 10 min, ^{32}P-labeled double-stranded F-DNA was added, and the ability of Ape1 to convert the longer AP site-containing DNA strand to a shorter oligonucleotide product was evaluated. As shown in Figure 2, Pb(II) and Fe(II) quantitatively exhibited the most pronounced inhibitory effects on Ape1 endonuclease activity in a concentration-dependent manner. Cd(II) also demonstrated an inhibitory effect, but only at the higher concentrations examined (starting at 10 μM). None of the other divalent metal compounds [i.e., As(III), Co(II), and Ni(II)] had a profound impact on Ape1 incision activity (Figure 2). Using the double reciprocal plot method of Porter et al. (1997), IC_{50} values for Cd(II), Fe(II), and Pb(II) were determined to be 26, 1.0, and 0.61 μM, respectively.

To explore whether the metal inhibition observed was universal to Ape1 nuclease activity, we examined the impact of the environmental metals on Ape1 3′- to 5′-exonuclease function (Wilson 2003). As anticipated, Ape1 exonuclease activity on a 1 nt gap substrate was similarly inhibited by Fe(II), Pb(II), and Cd(II), with no significant effect seen with the other metals (data not shown), suggesting a general (substrate-independent) inactivation of Ape1 catalytic activities.

Specific Inhibition of the Ape1-like Repair Proteins

In *E. coli* there are two major AP endonuclease proteins, ExoIII and EndoIV (Demple and Harrison 1994). These proteins represent distinct families, as there is

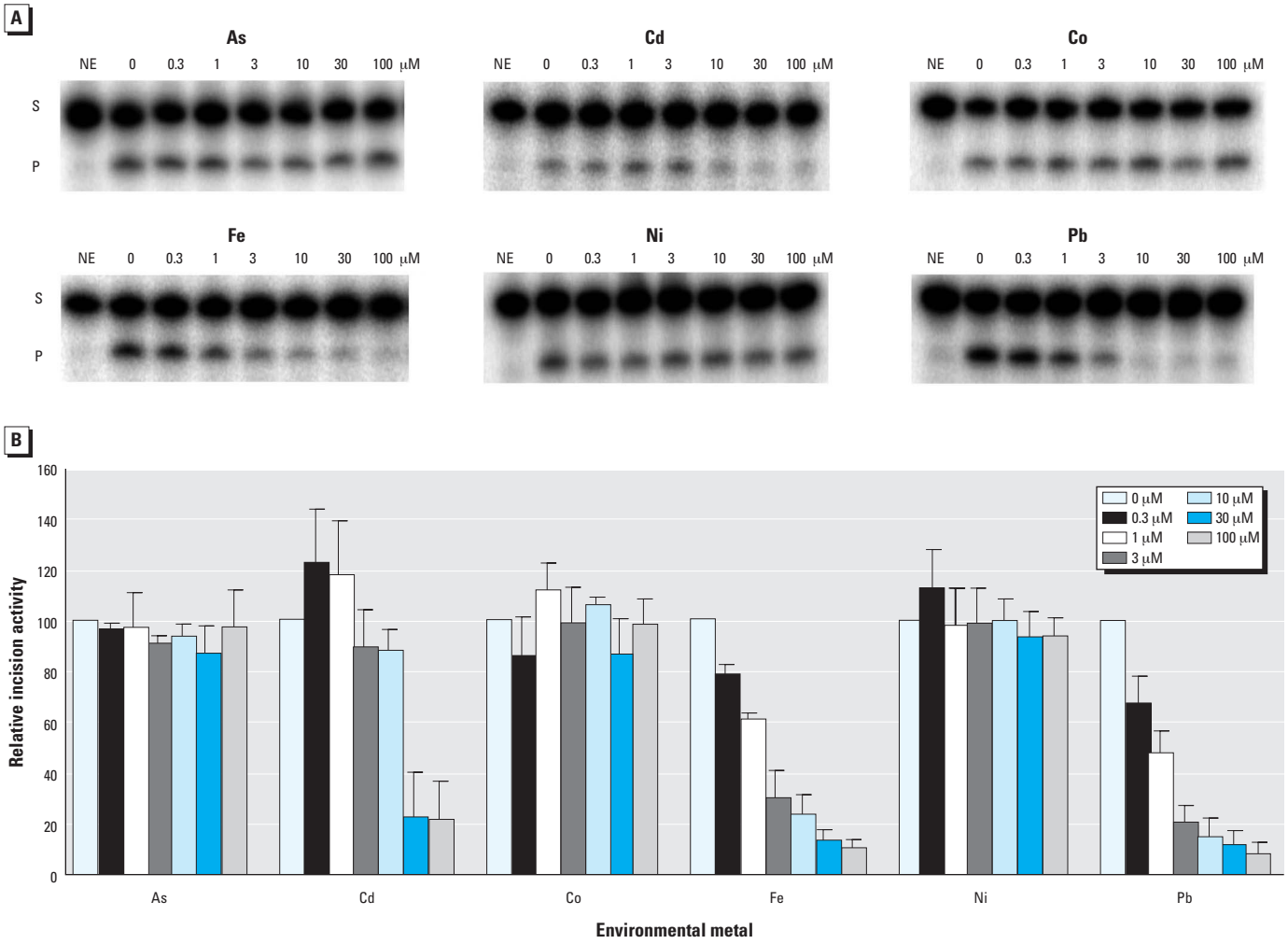

Figure 2. Effects of environmental metals on Ape1 incision activity. Abbreviations: NE, no enzyme control; E, enzyme + 1 mM $MgCl_2$, but no environmental metal; S, substrate; P, product. (*A*) Ape1 incision activity after exposure to As(III), Cd(II), Co(II), Fe(II), Ni(II), or Pb(II). Ape1 activity was monitored as described in "Materials and Methods." Notes (data not shown): Hydrochloric acid, which was used to dissolve the iron, had no effect (< 12%) on Ape1 incision activity at the highest concentration employed (100 μM). Ape1 exhibited an identical incision efficiency in $MgCl_2$- and MgAc-containing reaction buffers, indicating that the effects seen were the result of the lead ion and not the acetate. Moreover, lead acetate, when added to either $MgCl_2$- or MgAc-containing reactions, inhibited Ape1 incision activity. (*B*) Graphical presentation of the metal ion effects on Ape1 incision activity. Relative incision activity is based on a percentage comparison between the enzyme-only control standard incision and the individual incision reaction of interest. Values shown are relative Ape1 incision activities, normalized to the 0 μM, no environmental metal control (listed here as 100), and represent the average and standard deviation of at least three independent data points. Concentration of the environmental metals is indicated.

no primary amino acid sequence or structural homology between them (Mol et al. 2000a). Ape1 is a functional and structural homolog of ExoIII and belongs to this family of abasic endonucleases. To explore potential conservation of the metal-dependent inhibition seen with human Ape1, we examined the effects of As(III), Cd(II), Co(II), Fe(II), Ni(II), and Pb(II) on the AP site incision activity of the bacterial endonucleases (Figure 3). As anticipated, *E. coli* ExoIII showed a pattern of divalent metal inhibition similar to that observed with the Ape1 protein. Conversely, EndoIV was unaffected by the presence of any of the environmental metals. These results indicate a selective inhibition of the related ExoIII and Ape1 proteins by Cd(II), Fe(II), and Pb(II) and argue against an indirect (or nonspecific) effect of these metals.

Protein-Specific Inactivation

To further evaluate the specificity of the observed metal-dependent inactivation (Figure 2), addressing in particular whether the heavy metals simply electroplate DNA (i.e., bind along the phosphodiester DNA backbone) and interfere with Ape1 function indirectly, we examined the effects of Cd(II), Fe(II), and Pb(II) in the presence or absence of undamaged, nonspecific (chelating) DNA. Briefly, after incubation of Ape1 protein with an inhibitory metal, equimolar or 10-fold excess nonspecific competitor DNA (relative to the labeled F-DNA) was added simultaneously with radiolabeled abasic DNA substrate, and AP site incision was then measured. These studies revealed that undamaged DNA alone (i.e., in the absence of the heavy metal lead) had little (at 100 fmol) or a more significant (at 1 pmol) effect on Ape1 endonuclease activity (Figure 4). Regardless, nonspecific DNA did not abrogate the inhibitory effect of Pb(II) (Figure 4), suggesting that metal inactivation was protein-specific, consistent with the conclusion of the bacterial endonuclease studies presented above. Similar findings were obtained with Cd(II) and Fe(II) (data not shown).

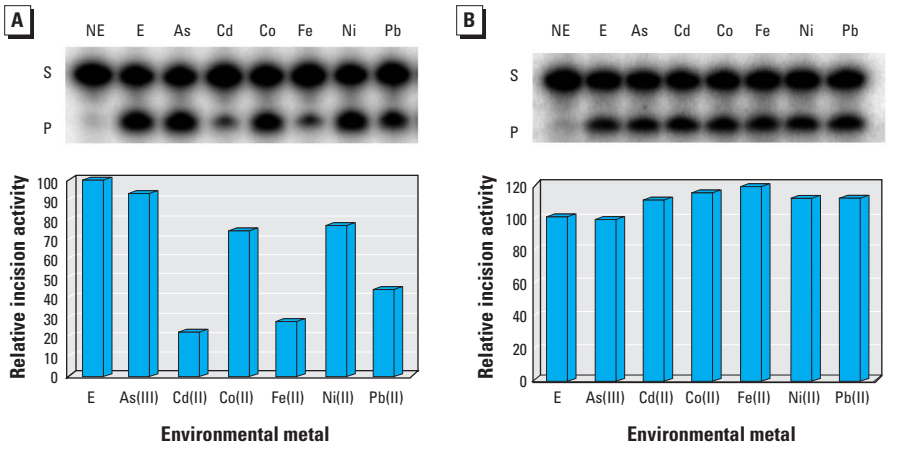

Figure 3. Effects of environmental metals on ExoIII (*A*) and EndoIV (*B*) AP site incision activity. Abbreviations: E, enzyme alone; NE, no enzyme control; P, product; S, substrate. In both panels a representative gel image (top) and the quantitative numbers (bottom) are shown. Relative incision activity is based on a percentage comparison between the enzyme-only control standard incision and the individual incision reaction of interest. Relative incision activities are normalized to the 0-μM no environmental control (listed here as 100) and represent the average of at least three independent data points. See "Materials and Methods" for details. Standard error for the values shown was ± 11%.

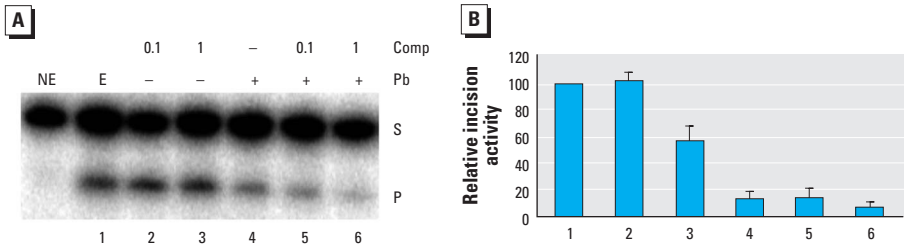

Figure 4. Heavy metal inhibition is not abrogated by nonspecific duplex DNA. Abbreviations: E, enzyme alone; NE, no enzyme control. (*A*) Competitor DNA does not affect lead inhibition. Ape1 is incubated without (E or –) or with lead (+, Pb), and then no (–), 0.1 pmol, or 1 pmol of nonspecific DNA (Comp) is added with radiolabeled F-DNA (0.1 pmol). The ability of Ape1 to convert substrate (S) to product (P) is then evaluated as described in "Materials and Methods." (*B*) Quantitation of competitor and lead effects on Ape1 incision activity. Relative incision activity is based on a percentage comparison between the enzyme-only control standard incision and the individual incision reaction of interest. Relative incision activity (*y*-axis) is shown, with standard deviations, for the six reaction conditions denoted in *A* and *B* (1–6). 1 = enzyme (E) control; 2 = Ape1 + 0.1 pmol competitor DNA; 3 = Ape1 + 1 pmol competitor; 4 = Ape1 + 10 μM Pb(II); 5 = Ape1 + 10 μM Pb(II) + 0.1 pmol competitor; 6 = Ape1 + 10 μM Pb(II) + 1 pmol competitor.

Specificity of Metal Inhibition in Whole-Cell Extracts

To more broadly assess the specificity of (and the effects of nonspecific proteins on) the metal-dependent inhibition reported above, we determined the impact of As(III), Cd(II), Co(II), Fe(II), Ni(II), and Pb(II) on AP endonuclease, 1 nt gap polymerase fill-in, 5′-flap structure-specific endonuclease, and nick ligation activities in whole-cell extracts (see substrates depicted in Figure 1B and assays described in Figure 5 legend). We reasoned that whole-cell extracts would better mimic the *in vivo* environment, as they contain a representative mix of proteins. We examined the above enzymatic activities, as they are central to many DNA metabolic processes, including BER (Kelley et al. 2003). As shown in Figure 5, only AP endonuclease activity was markedly inactivated by Cd(II), Fe(II), and Pb(II). This finding emphasizes that these heavy metals do not simply display universal, nonspecific inhibition of enzymatic processes and may suggest that Ape1 (which comprises > 95% of the total cellular AP site incision activity) (Demple et al. 1991) is selectively inactivated by Cd(II), Fe(II), and Pb(II) *in vivo*.

Discussion

We demonstrate here that Cd(II), Fe(II), and Pb(II), three potential carcinogens (Huang 2003; Silbergeld 2003; Waalkes 2003; Waisberg et al. 2003), can selectively inactivate Ape1 repair activity *in vitro* (Figure 2), with IC_{50} (half-maximal inhibitory concentration) values of 26, 1.0, and 0.61 μM, respectively. To our knowledge, whereas Cd(II) has been shown to inhibit a number of DNA repair enzymes, our studies are the first to identify a specific DNA repair protein target for lead. Because of their accumulation in the choroid plexus (the blood–cerebrospinal fluid barrier), cadmium and lead have also been connected with neurotoxicity and neurological disorders, which are often associated with elevated oxidative stress and/or inefficient repair responses (Zheng 2001).

Although Fe(II) is thought to elicit most of its deleterious impact via metal-catalyzed free radical production (Kasprzak 2002), a concomitant inhibition of a key oxidative DNA repair protein such as Ape1

could potentiate its harmful effects. Iron is rarely found in the environment in a +2 state; however, Fe(II) can accumulate in a mobile or labile form during conditions of iron overload or after disruption of cellular homeostasis. Notably, iron misregulation (or overload) in the brain plays an important role in neuronal death in some neurodegenerative disorders, such as Alzheimer, Parkinson, and Huntington disease, as well as Hallervorden-Spatz syndrome (Ke and Ming 2003). In total, evidence suggests that certain environmental metals elicit their carcinogenic and/or neurodestructive effects by inducing oxidative damage (particularly true for iron via the Fenton reaction) and inhibiting repair processes (Hartwig et al. 2002; Hartwig and Schwerdtle 2002; Hayes 1997; Kasprzak 2002).

Pb(II) levels have been observed at > 20 μg/dL (or ~0.6 μM) in the blood of health-impaired individuals (Silbergeld 2003) and as high as 1–10 μM in certain occupationally exposed workers (Hayes 1997). If a comparable concentration is attained in cells, then based on the IC_{50} value of Pb(II), ≥ 50% of Ape1 activity would be inhibited. It is noteworthy that a majority of the intracellular lead has been found in the nucleus (Hitzfeld and Taylor 1989). For Cd(II) the amount of free metal is normally thought to be negligible because of its very high affinity for metallothioneins. However, cadmium ions may be transferred from one protein to another by exchange reactions if relative affinities permit (Klaassen et al. 1999). During conditions of oxidative stress, free Cd(II) levels may reach *in vivo* concentrations in the millimolar range (Zharkov and Rosenquist 2002). Intracellular iron is either tightly bound within iron-containing proteins (e.g., those factors with iron–sulfur centers) or more transiently associated with low-molecular-weight (LMW) ligands (Huang 2003). In these latter complexes, iron is easily exchangeable and thus bioavailable for its essential functions as well as adverse effects, including metal-catalyzed free radical production and promiscuous enzyme binding. The estimated concentration of iron bound to the LMW ligands (the so-called chelatable iron) is 1–10 μM in rodent and human cells (Petrat et al. 2002). Given the metal concentrations at which Ape1 activity is inhibited (excluding localized accumulation) and the selective inactivation seen in cell extracts (Figure 5), it seems reasonable to conclude that Cd(II), Fe(II), and Pb(II) may promote their harmful effects through inhibition of Ape1 repair function.

Significantly, both Cd(II) and Pb(II) display co-genotoxic effects when combined with methyl methanesulfonate, *N*-methyl-*N*-nitrosourea, or *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, alkylating agents that generate significant levels of BER-type DNA intermediates, including abasic lesions (Fatur et al. 2003; Hartwig 1994; Roy and Rossman 1992). Thus, because reduced Ape1 repair capacity (of 50%) has been correlated with survival and cancer proneness (Meira et al. 2001), we postulate that certain environmental metals such as Cd(II), Fe(II), and Pb(II) elicit their co-mutagenic effects in part by inactivating (or depleting) Ape1 repair activities. In support of the idea of targeted inhibition of Ape1 *in vivo*, we found that Cd(II), Fe(II), and Pb(II) [but not As(III), Co(II), or Ni(II)] specifically inhibited AP endonuclease activity in whole-cell extracts but did not dramatically affect other steps of repair, such as single nucleotide gap-filling, 5′-flap endonuclease, and nick ligation activities (Figure 5). These findings also speak to the selectivity of these heavy metals for the Ape1 repair protein in the context of a protein milieu. Cellular strategies are now being devised—keeping in mind the direct DNA-damaging (oxidizing) effects of certain environmental metals (Hayes 1997; Kasprzak 2002) and the apparent requirement of Ape1 for viability (Ludwig et al. 1998; Meira et al. 2001; Xanthoudakis et al. 1996)—to evaluate whether the metal-induced cytotoxic and/or mutagenic outcomes noted above are at least in part Ape1 dependent.

Most transition metals bind acidic, sulfhydryl, or histidine residues within target proteins. Lead, in particular, exhibits high affinity for Cys–His zinc-binding motifs (Silbergeld 2003). Although we cannot state with certainty the mechanism by which Cd(II), Fe(II), or Pb(II) [three metals that have divergent coordination chemistries and different ligand preferences (Lippard and Berg 1994)] inactivates Ape1, both X-ray crystallography and NMR spectroscopy studies found that lead can occupy

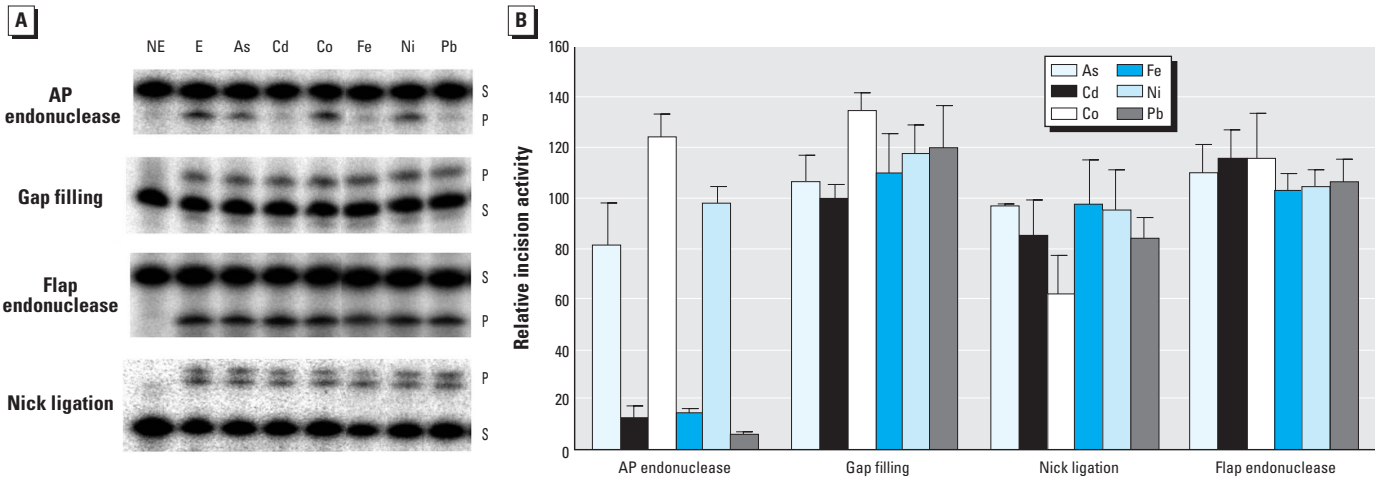

Figure 5. Metal inhibition in whole-cell extract assays. Abbreviations: E , enzyme alone; NE, no enzyme control; P, product; S, substrate. (*A*) Cd(II), Fe(II), and Pb(II) selectively inactivate total AP endonuclease activity. Whole-cell extracts were prepared, and the indicated DNA metabolic activity was measured in the presence or absence of the noted heavy metal as described in "Materials and Methods." See Figure 1B for substrates. For gap filling, single nucleotide extension of the 5′-end-labeled 15P primer was monitored. For flap endonuclease activity, conversion of the 29-mer 34(10)flap oligonucleotide to a 10-mer DNA product was examined. Nick ligation was measured by determining the conversion of the radiolabeled 15P oligonucleotide to the full-length, ligated 34-mer (or its 33 nucleotide degradation product). The two products in the nick ligation assays presumably arise from ligation, followed by 3′- to 5′-exonuclease degradation. (*B*) Graphical presentation of the metal ion effects on the various metabolic activities. Values shown are relative activities, compared with the 0 μM, no environmental metal control (designated 100), and represent the average and standard deviation of at least three independent data points. Concentration of the environmental metals (indicated) was 100 μM.

two potential divalent metal binding sites within the Ape1 active site (Beernink et al. 2001; Lowry et al. 2003). In particular, lead associates with residues in Ape1 essential for enzymatic activity, most notably His309, in a manner distinct from that seen with zinc-binding proteins. Current evidence therefore suggests that inactivation of Ape1 is mediated by a unique and specific interaction of the inhibitory metal with conserved active site residues that in turn disrupts the metal-dependent (magnesium-dependent) catalytic reaction. Consistent with this notion, Ape1 and the homologous ExoIII protein exhibit a similar metal-dependent inactivation profile, whereas the structurally distinct AP endonuclease EndoIV was not affected (compare Figures 2 and 3), implying a conserved and targeted effect of Cd(II), Fe(II), and Pb(II) on the Ape1-like repair proteins. Furthermore, competition assays using nonspecific DNA argue against an indirect effect of the metal ions, such as electroplating of DNA (Figure 4), and suggest a protein-specific event. Finally, Ape1 DNA binding was not dramatically altered by the presence of the various heavy metals [excluding Fe(II), where a smear was observed] in gel mobility shift assays (unpublished observations), suggesting disruption of the enzymatic step specifically. While detailed structure-function studies are under way to precisely define the mechanism of inhibition, the experiments here suggest a novel means by which these prevalent environmental metals may elicit their harmful physiological effects.

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